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Pathologic Cuticular Changes of Winter Impoundment Shell Disease Preceding and During Intermolt in the American Lobster, *Homarus americanus*

ROXANNA M. SMOLOWITZ, ROBERT A. BULLIS, AND DONALD A. ABT

Laboratory for Marine Animal Health, School of Veterinary Medicine, University of Pennsylvania, Marine Biological Laboratory, Woods Hole, Massachusetts, 02543

Abstract. Cuticular lesions from twenty-four market sized lobsters (*Homarus americanus*) with winter impoundment shell disease were examined. Histological descriptions of cuticular lesions were correlated with the substage of molt for each lobster, because cuticle components and inflammatory mechanisms vary in each. A lesion severity grading system was developed and applied to four specific substages of the five-stage (A–E) molting cycle. Lesions present in substage C_4, in which the membranous layer is deposited, and D_0 (passive premolt) were divided into five grades, ranging from mild erosions (Grade I) to severe ulceration (Grade V) of the cuticle. Cuticular lesions from lobsters in C_4/D_0 were compared with cuticular lesions from lobsters in substages C_2/C_3. Defensive mechanisms exhibited by animals in all substages were epicuticle deposition, melanization, inflammatory cell infiltration, and pseudomembrane formation. In addition, animals in C_4 and D_0 showed proliferation of the membranous layer in affected foci. The lesion grading scheme presented in this paper can be used to describe and compare both inter- and intraspecies crustacean shell lesions.

Introduction

After their capture, and until their sale, many American lobsters, *Homarus americanus*, are held in tidal impoundments. These impoundment populations can be maintained for up to six months, a period sufficient to span closed seasons and times of adverse weather. The impoundments, built by damming off coves or suitable sections of beach, must be located where the rise and fall of the tides are great enough to ensure adequate flushing of the pound. Ideally, impoundments should be cool in summer, seldom freeze in winter, and be in an area of relatively high and constant inshore salinity. Conditions in a pound primarily depend on the number and health of the lobsters impounded, the quality of the water supply, and the extent to which it is replaced at high tide. Even in excellent locations, unfavorable conditions may develop in pounds, particularly during neap tides (McLeese and Wilder, 1964).

A commonly recognized problem of lobsters held in impoundments during the winter months is an increase in shell disease. This unsightly condition, characterized by blackened (melanized) erosions into the cuticle, affects marketability and can spread through a population leading to widespread losses and decreased profitability. Winter mortalities due to shell disease have historically been a particular problem in Nova Scotia (Hess, 1937; Taylor, 1948; Malloy, 1978; Bullis, 1989; Getchell, 1989). Previous reports provide minimal information about the inflammatory mechanisms and pathogenesis of this disease, especially with regard to the differences apparent during various stages of the molting cycle. Malloy (1978) briefly described progressive cuticular erosions resulting from the establishment of mixed populations of bacteria, with chitinolytic ability, within the lesions. But he did not describe the inflammatory mechanisms or the effects of the molting cycle on the histological appearance of the erosions. Any description of progressive cuticular lesions must be related to the histological appearance of the normal cuticle during the several phases of the molting cycle. Such information is essential to an understanding of lesion development and its effect upon the animals.

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Table I

Histological appearance of [illegible] during stages of the normal molting cycle [illegible] lobsters (Aiken, 1980; Travis, 195[illegible] 1962)

Stage of molt	Changes in the integument	Layers of cuticle present*
Stage A	Mineralization of the postecdysial cuticle	Epicuticle[1] Exocuticle[1]
Stage B	Striated endocuticle is deposited	Epicuticle[1] Exocuticle[1] Striated endocuticle[1]
Stage C		
Substage C_1	Decreased height of cuticular epithelium	Epicuticle[1] Exocuticle[1] Striated endocuticle[1]
Substages C_2 and C_3	Thin lamellar endocuticle is deposited in the carapace	Epicuticle[1] Exocuticle[1] Striated endocuticle[1] Thin-lamellar-endocuticle[1]
Substage C_4	Intermolt. Membranous layer is deposited	Epicuticle[1] Exocuticle[1] Striated endocuticle[1] Thin-lamellar-endocuticle[1] Membranous layer[1]
Stage D		
Substage D_0	Passive premolt. Transition period from intermolt to premolt.	Epicuticle[1] Exocuticle[1] Striated endocuticle[1] Thin-lamellar-endocuticle[1] Membranous layer[1]
Substage D_1	Retraction of the epidermis from the cuticle with dissolution of the inner portion of the membranous layer (apolysis) and deposition of a new epicuticle	Old cuticle[1] Apolytic space Epicuticle[2]
Substage D_2	Deposition of exocuticle in the new cuticle	Old cuticle[1] Apolytic space Epicuticle[2] Exocuticle[2]
Substage D_3	Extensive reabsorption of minerals from the endocuticles of the old cuticle	Epicuticle[1] Exocuticle[1] Dissolution of the endocuticles[1] Apolytic space Epicuticle[2] Exocuticle[2]
Stage E	Ec[illegible]	Epicuticle[2] Exocuticle[2]

* The superscript[1] identifies those cuticular layers (old cuticle) which will be eliminated at ecdysis (Stage E). The superscript[2] designates cuticular layers that will remain as part of the postecdysial (new) cuticle.

The crustacean molting cycle is divided into five major stages (A through E) (Table I), with most stages further divided into substages (Travis, 1955; Dennell, 1960; Skinner, 1962; Aiken, 1980). In this study, lesions occurring principally in four substages of the molt cycle were examined (C_2, C_3, C_4, and D_0). Substages C_2 and C_3 (of Stage C) are not readily divisible by histological examination in homarids, but they span the time of thin-lamellar-endocuticle deposition (Fig. 1) (Table I).

In C_4, passive intermolt, the normal decapod cuticle is made up of four layers; epicuticle, exocuticle, endocuticle, and membranous layer (Fig. 2) (Table I) (Aiken, 1980). The endocuticle is divided into two parts, the striated endocuticle and the thin-lamellar-endocuticle. In homarids, the thin-lamellar-endocuticle does not contain vertical striations but does contain calcium; but both of these are features of the striated endocuticle. The membranous layer is a thin, dense band that is deposited during C_4 internal to the thin-lamellar-endocuticle by epithelial cells. Passive premolt, D_0, is described as a time of slow, gradual tran-

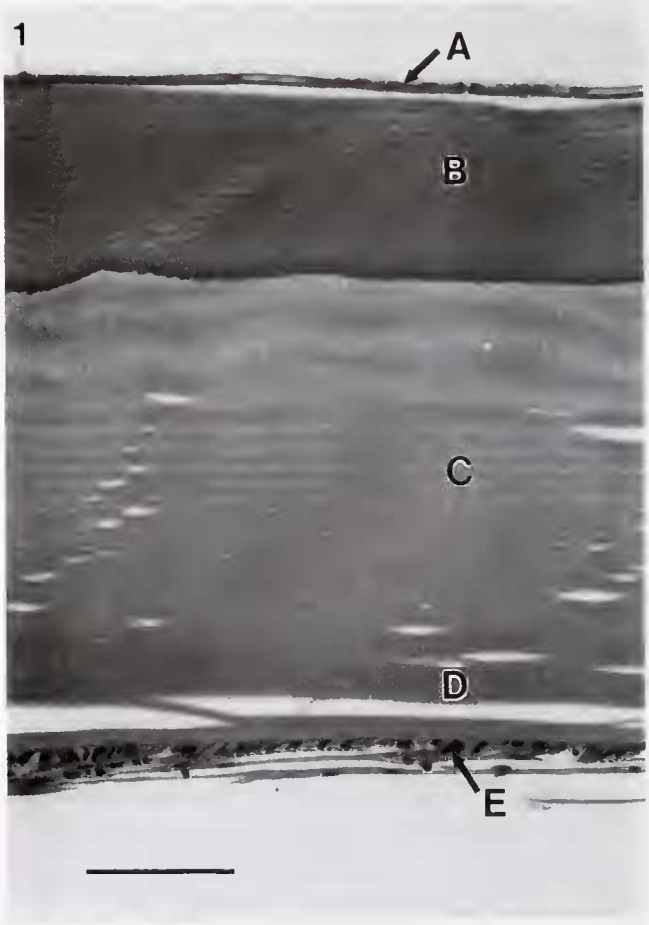

Figure 1. Normal cuticle of a lobster in C_2/C_3. Epicuticle (A), exocuticle (B), striated endocuticle (C), thin-lamellar-endocuticle (D), cuticular epithelium (E). Bar represents 60 μm.

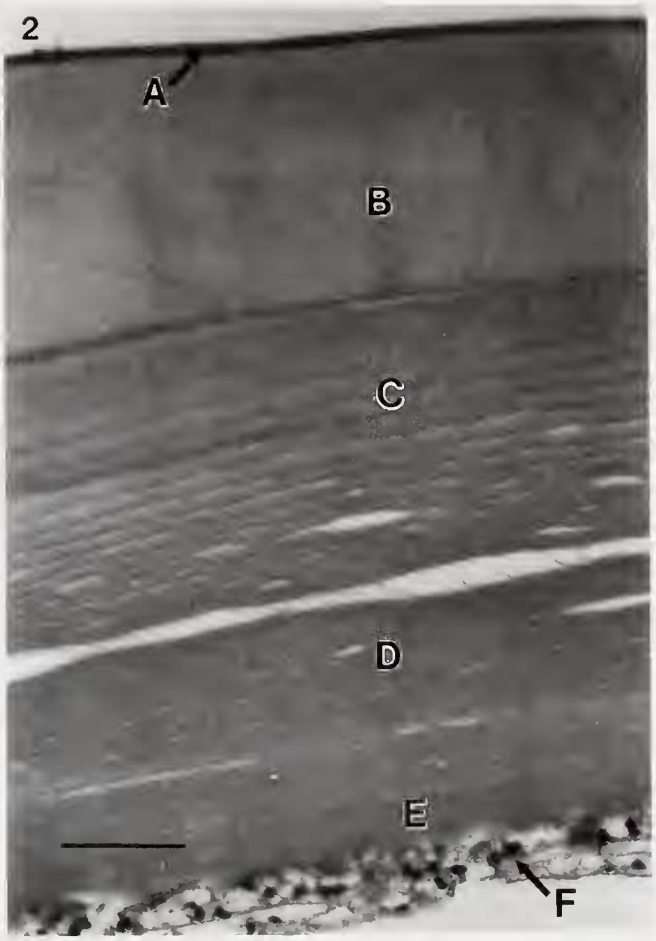

Figure 2. Normal cuticle of a lobster in C_4/D_0. Epicuticle (A), exocuticle (B), striated endocuticle (C), thin-lamellar-endocuticle (D), membranous layer (E), cuticular epithelium (F). Bar represents 60 μm.

sition between C_4 and D_1. The inner portion of the membranous layer dissolves in D_1 to form a fracture line between the old cuticle and the new (apolysis) (Aiken, 1980). Some authors include the membranous layer as part of the thin-lamellar-endocuticle (Dennell, 1960; Aiken, 1980). The function of the membranous layer before dissolution (and that which the thin-lamellar-endocuticle shares in homarid lobsters) is exhibited by the broken-cuticle test, in which the flexible membranous layer prevents a clean breakage of the cuticle and holds the two edges together (Aiken, 1980). Substage C_4, and D_0, which follows it, are histologically indistinguishable in the general cuticle. Together, the duration of these two substages makes up approximately 56% of the molt cycle (Aiken, 1980).

In this paper we describe the histological changes associated with the progressive cuticular erosions in lesions of winter impoundment shell disease as seen in a non-randomly selected group of 24 lobsters held in a winter impoundment facility for several months. Lobsters were predominately in either C_2/C_3 or C_4/D_0 of the molting cycle, and descriptions were therefore limited to these substages.

Materials and Methods

Twenty-four market size lobsters (450–500 g) were obtained through Paul's Lobster Co. (Boston, Massachusetts) from their winter impoundment on Gran Manan Island, Nova Scotia, Canada. Lobsters were collected during a disease outbreak and were chosen to provide a spectrum of lesions from grossly normal to severely eroded. The severity of the cuticular lesions on each lobster varied, but the number of foci and the severity of the erosions were graded in each individual, with a few animals containing numerous deep erosions.

Winter impoundment associated cuticular lesions were prepared for histological study. Tissues were fixed in 10% formalin in seawater (3.7% active formaldehyde), decal-

Figure 3. Dorsal view of *Homarus americanus* showing severe signs of shell disease characterized by diffuse pitting erosions (arrows) of the chelipeds, carapace, and abdominal segments.

Figure 4. Ventral view of *Homarus americanus* showing the characteristic hyperpigmentation (arrow) usually associated with naturally acquired abrasions and scratches.

Figure 5. Traumatic cracking (arrow) of the exoskeleton resulting from aggressive behavior. Crushing lesions are common in impounded lobster populations.

cified in formic acid/sodium citrate, embedded in paraffin, and stained with hematoxylin and eosin using standard techniques. The nomenclature proposed by Aiken (1980) was used to describe the layers of the cuticle. These lobsters were subsequently identified histologically as being in substages C_2/C_3 or C_4/D_0 of the molting cycle. In this paper, cuticular lesions from lobsters in substages C_2 and C_3 are described together, as are lobsters in substages C_4 and D_0. After examination, representative samples were selected and a grading system was developed based on the depth and severity of the erosions.

Results

Gross examination of affected animals revealed diffuse pitting erosions covering the entire exoskeleton, but most prominent on the carapace and dorsal abdominal segments (Fig. 3). The appearance of individual erosions varied greatly in the amount of melanization (abnormal black discoloration) and the depth of the erosion. No attempt was made in this study to correlate the gross appearance of individual erosions with their histological appearance. Other types of shell lesions noted were: lesions of the ventral surface characterized by linear foci of hyperpigmentation associated with abrasions and scratches (Fig. 4); and crushing injuries manifested by cracking of the exoskeleton and severe blackening of underlying tis-

Table II

Shell disease grading system for homarid molting substages C_4/D_0 and C_2/D_3

Grade I. Epicuticular erosion.
Grade II. Erosion into the exocuticle.
Grade III. Erosion into the striated endocuticle and the thin-lamellar-endocuticle.
Grade IV. Erosion into the pseudomembrane.
Grade V. Total erosion of the cuticle and ulceration of the underlying cuticular epithelium.

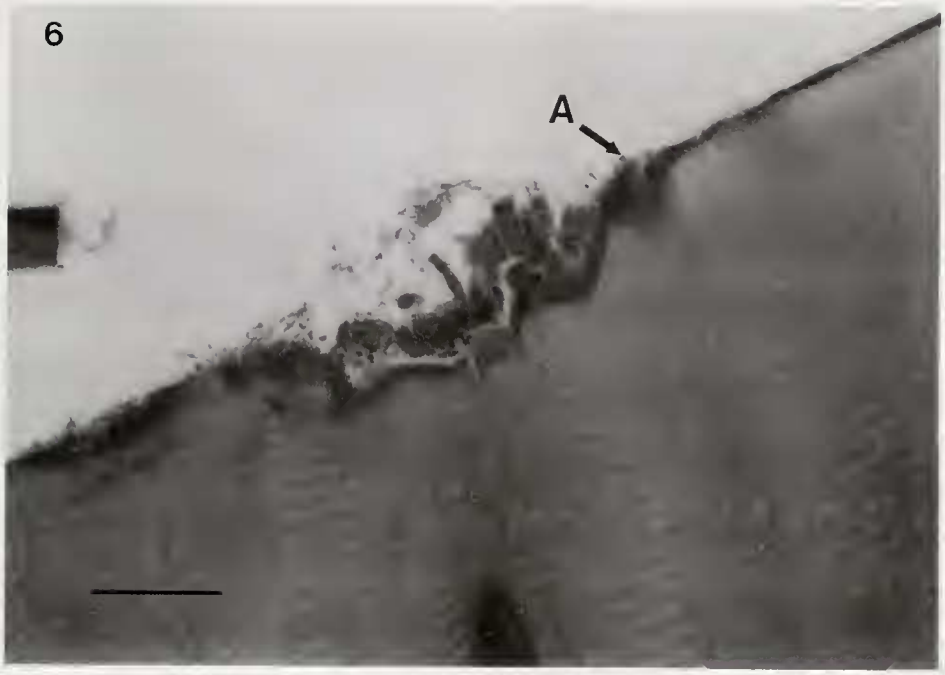

Figure 6. C_4/D_0. Grade I. Erosion of the epicuticle (A) and colonization of the setal pit by various organisms. Bar represents 44 μm.

sues (Fig. 5). These last two types are representative of other common lesions present in lobster cuticles both in impoundments and in the wild. These two lesion types will be described in subsequent papers.

Histopathology of shell lesions

Because the majority of the homarid molt cycle is spent in C_4/D_0, lesions will be first described for these substages, followed by comparative descriptions of C_2/C_3 lesions.

Histopathology of shell lesions from lobsters in C_4/D_0 (Table II)

Histological examination of cuticles from lobsters in the final intermolt stage (C_4/D_0) demonstrated a membranous layer present in both inflamed and normal cuticle. The cuticular lesions were divided into five progressive grades of severity and were described as follows:

Grade I. Epicuticular erosion in the perisetal cuticle (Fig. 6) and tegmental pore cuticle. Seta are normally present within shallow, regular pits in the cuticle. Erosions through the epicuticle appeared to first occur at foci in the shoulders and sides of setal pits and were characterized by necrosis and melanization. Occasionally there was melanization of the innervating neuronal core within the cuticular canals in the epicuticle and exocuticle. Only rarely was cuticle around setal pores necrotic and melanized. In a few affected foci, a very mild increase in the thickness of the outer layer of the epicuticle was seen. The dermal tegmental glands in these animals were large and prominent, indicating the early portion of the C_4/D_0 period. In late C_4/D_0, dermal tegumental glands were greatly reduced in size. The flora within the setal pits were mixed but often were dominated by filamentous algae. The underlying cuticular epithelium was mildly hypertrophic.

Grade II. Erosion into the exocuticle (Fig. 7). Erosions extended into the exocuticle with necrosis, and loss of cuticular matrix, resulting in the formation of shallow craters in the carapace. Mild to moderate melanization of the exposed exocuticular border occurred. Tissue decalcification, however, produced variably bleached shades of melanin (Smolowitz *et al.*, 1992) associated with the inflammatory response that consisted of granulocytes, agranulocytes, and semigranulocytes. Epidermal hypertrophy, in varying amounts, was evident at the base of the erosions. There was a mild influx of granulocytes and agranulocytes with occasional diapedesis of these inflammatory cells between epidermal cells. In some animals, significant thickening of the inner thin-lamellar-endocuticle/membranous layer was noted, especially in Grade II lesions adjacent to more severely graded foci. Grade II appeared rarely in Stage C_4/D_0 of the molt cycle and most Grade II lesions were adjacent to Grade III lesions.

Grade III. Erosion into the striated endocuticle and the thin-lamellar-endocuticle (Figs. 8, 9). Most erosions examined in this grade extended into the striated endocuticle

Figure 7. C_4/D_0, Grade II. Erosion into the exocuticle with melanization (A). Thickened thin lamellar/membranous layer (B). Mildly hypertrophic epithelium (C). Focus of inflammatory cell accumulation between the outer thin-lamellar-endocuticle and the inner thin-lamellar-endocuticle/membranous layer (D). Bar represents 100 μm.

and only rarely into the outer thin-lamellar-endocuticle. Melanization of the surface of these erosions was moderate to severe and occasionally extended vertically into the thin-lamellar-endocuticle. The inflammatory cell population was increased from Grade II, and diapedesis of cells through the epidermis and into the cuticular canals was noted. Puckering of the membranous layers occasionally formed pockets predominately containing granulocytes. These pockets often appeared to be associated with cuticular canals. Accumulations of inflammatory cells were present multifocally at the junction of the striated endocuticle with the thin-lamellar-endocuticle, between layers of the thin-lamellar-endocuticle, at the junction of the thin-lamellar-endocuticle with the membranous layer and within layers of the membranous layer. In some instances, loosely attached pseudomembranes were formed in these same areas. The pseudomembranes, varying between 5 to 25 cells in thickness, were composed predominately of layers of spindle shaped, transformed inflammatory cells primarily oriented parallel to the surface epithelium. Other non-transformed inflammatory cells were found in lesser numbers within the pseudomembranes.

In several inflamed foci, the normal sequential and orderly change in the histological appearance between the thin-lamellar-endocuticle and the membranous layer no longer was present. Instead, an abnormal section of cuticle indistinctly began at the inner side of the thin lamellar cuticle and continued to the surface of the cuticular epithelium. This unusual section of cuticle appeared histologically to be composed of repetitive alternations of the thin-lamellar-endocuticle and the membranous layer. In such inflamed areas, this combination of inner thin-lamellar-endocuticle and membranous layer had increased in depth, sometimes greatly, when compared to adjacent unaffected cuticle.

Rarely in the inflamed areas, cuticular pearl formation was present in the dermis (Fig. 9). These structures were composed of thin-lamellar-endocuticle secreted by dermal nests of displaced cuticular epithelial cells. Such nests were formed by focal invagination or detachment of cuticular epithelial cells. Multifocal, mild to severe, tegmental adenitis and setal cell inflammation were characterized predominately by encapsulation and melanization. The cuticular epithelium was hypertrophic and often vacuolated at its cuticular surface.

Grade IV. Erosion into the pseudomembrane (Fig. 10). Lesions of this grade were characterized by erosions through the striated endocuticle and outer layers of the thin-lamellar-endocuticle, thus exposing a pseudomembrane which protected the otherwise exposed inner combined thin-lamellar-endocuticle/membranous layer below. The pseudomembrane appeared as described in Grade III, but varied between 25 to 150 cells in thickness, and the necrotic surface of the pseudomembrane was melanized to varying depths. The inner thin-lamellar-endocuticle/membranous layer below the pseudomembrane was often thickened. Other dermal changes were as described in Grade III.

Grade V. Total erosion of the cuticle and ulceration of the underlying cuticular epithelium (Fig. 11). Lesions of Grade V were focal necrosis and sloughing of the cuticle with ulceration of the epithelium. The resulting ulcers were covered by a thick pseudomembranous crust consisting of alternating layers composed of predominately horizontally aligned, transformed inflammatory cells between layers of edematous necrotic tissue. One to two layers of transformed inflammatory cells were firmly attached to, and formed the surface of, the dermis. Hyperplastic and hypertrophic epithelium were present at the edges of the ulcers. The severe dermatitis was characterized superficially by a great influx of inflammatory cells and by transformed inflammatory cells randomly oriented to-


8a
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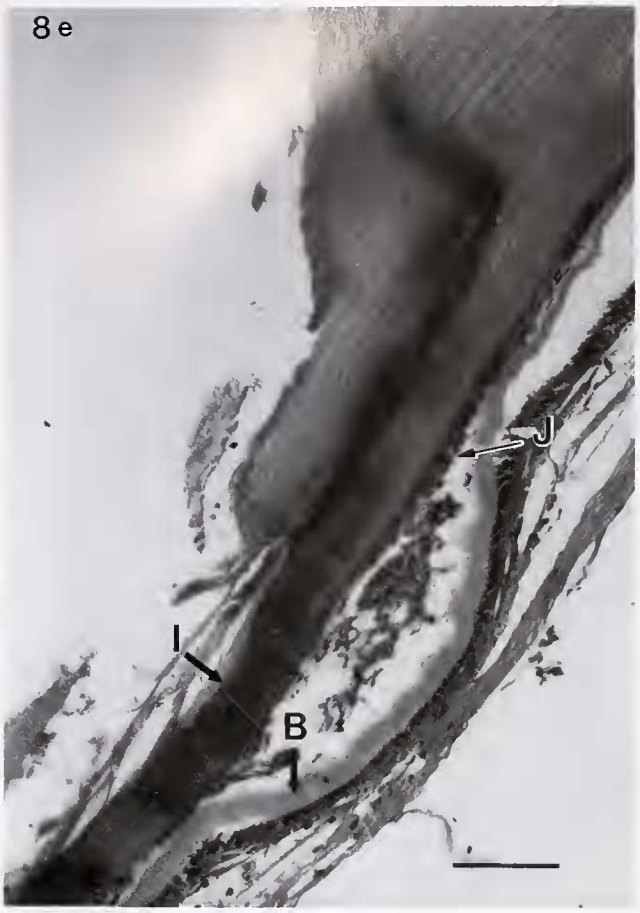

Figure 8. C_4/D_0, Grade III. 8a–d: Erosion into the striated endocuticle with melanization (A). Proliferation of the inner thin-lamellar-endocuticle/membranous layer (B). 8a. Puckering of the thin-lamellar-endocuticle/membranous layer with accumulation of granulocytes in pockets (C). Diapedesis of inflammatory cells (D) through hypertrophic epithelium (E) Bar represents 100 μm. 8b. Focal large accumulation of inflammatory cells (F) between the outer thin-lamellar-endocuticle and the inner thin-lamellar-endocuticle/membranous layer. Bar represents 60 μm. 8c. Accumulation of granulocytes in pockets associated with cuticular canals between layers of the thin-lamellar-endocuticle (G). Bar represents 100 μm. 8d. Molted cuticle showing the ecdysial separation line (H). Bar represents 230 μm. 8e. Increased depth of the inner thin-lamellar-endocuticle/membranous layer (B). Erosion into the outer thin-lamellar-endocuticle with melanization (I). Formation of a pseudomembrane between the outer thin-lamellar-endocuticle and the inner thin-lamellar-endocuticle/membranous layer (J) in late grade III. Bar represents 100 μm.

ward the surface layers of transformed cells. Within the deeper dermis, myoblastic proliferation was present as evidenced by bundles of myoblastic cells occurring at angles (somewhat perpendicular) to the surface. Melanized encapsulations (granulomas), many of which represented foci of tegmental gland and setal cell inflammation as well as thrombi within the open circulatory system, were scattered throughout the dermis. Edema, vascular dilation, margination of inflammatory cells in vessels, and multifocal severe necrotizing myositis of the underlying muscles were also noted.

The individual from the study group of 24 lobsters that demonstrated with Grade V lesions was in late D_1/early D_2 of the molt cycle as determined by examining areas of the cuticle away from the lesions. However, the description of the lesion is completely applicable to C_4/D_0 because: as evidenced by gross observation, the lesion initially developed during the intermolt or early premolt time period (before new cuticle developed); there was a lack of new cuticle in the area of the lesion; and substages D_1 and D_2 occur very quickly and would not have allowed sufficient time for this lesion to develop.

Pathology of shell lesions from lobsters in C_2/C_3 (Table II)

In C_2/C_3, cuticular lesions were also divided into five grades that were comparable to those in C_4/D_0. In C_2/C_3 the membranous layer had not yet been formed and proliferation of the inner thin-lamellar-endocuticle/membranous layer had not occurred.

Grade I. Epicuticular erosion in the perisetal cuticle and tegmental pore cuticle. In contrast to C_4/D_0, dermal tegmental glands were large and prominent throughout these substages of the molting cycle. Other changes, including colonization of the setal pits by a mixed flora often predominated by filamentous algae (Fig. 12), were as described for Grade I of C_4/D_0.

Grade II. Erosion into the exocuticle (Fig. 13). The eroded cuticular surface appeared as in Grade II of C_4/D_0. Epithelial hypertrophy with diapedesis of inflammatory cells was noted as above, but interestingly and characteristically, this stage of molt exhibited accumulations of inflammatory cells between the epithelium and the base of the cuticle. These foci were from two to four cells thick and consisted of a mixture of inflammatory cells, often predominated by granulocytes.

Grade III. Erosion into the striated endocuticle and the thin-lamellar-endocuticle (Fig. 14). Early Grade III lesions were characterized by erosions into the upper levels of the striated endocuticle. However, at the epithelial-cuticular border, an accumulation of a mixed population of inflammatory cells formed a pseudomembrane populated predominately by transformed cells oriented parallel to the surface of the epithelium and base of the cuticle. This pseudomembrane was from 5 to 30 cells thick. The cuticular epithelium was hypertrophic and hyperplastic and displaced ventrally from the cuticle to a position below the pseudomembrane. The inner thin-lamellar-endocuticle/membranous layer was not present between the pseudomembrane and the epithelium. Tegmental adenitis was present multifocally in the dermis.

Grade IV. Erosion into the pseudomembrane. This grade was not applicable to C_2/C_3 because the membra-

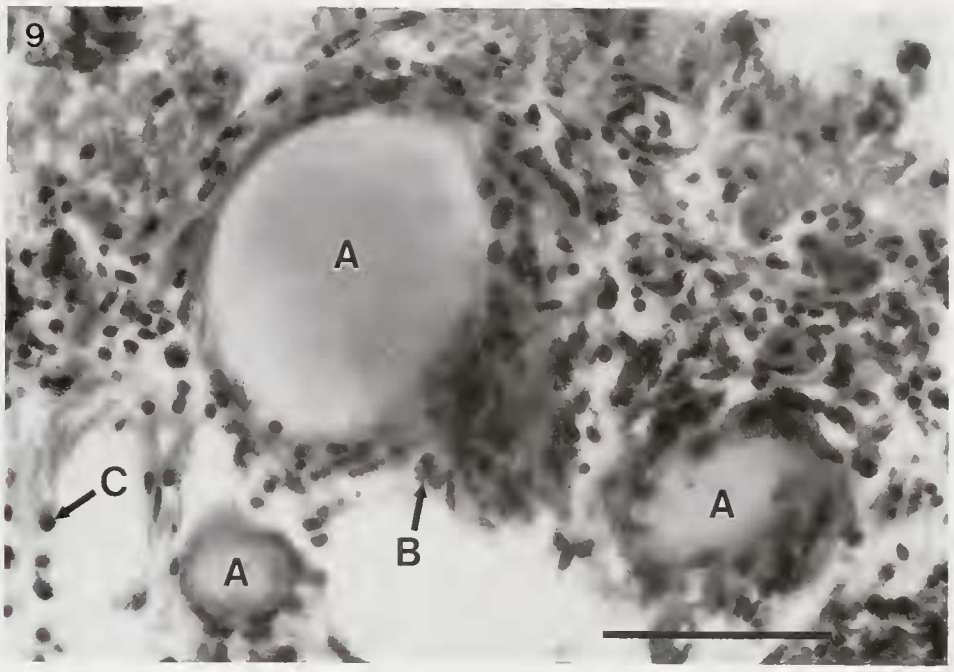

Figure 9. C_4/D_0, Grade III. Cuticular pearls (A) in the dermis of inflamed cuticle containing granulocytes (B) and agranulocytes (C). Bar represents 64 μm.

nous layer was not yet formed and there was no proliferation of the inner thin-lamellar-endocuticle/membranous layer as in C_4/D_0.

Grade V. Total erosion of the cuticle and ulceration of the underlying cuticular epithelium. No Grade V lesions were seen from lobsters in C_2/C_3 of the molt cycle but would be very similar to those of Grade V in C_4/D_0 lobsters. Evidence for this conclusion is provided by examination of a severe shell lesion from an animal in C_2/C_3 of the molt cycle that showed a late grade III/early grade

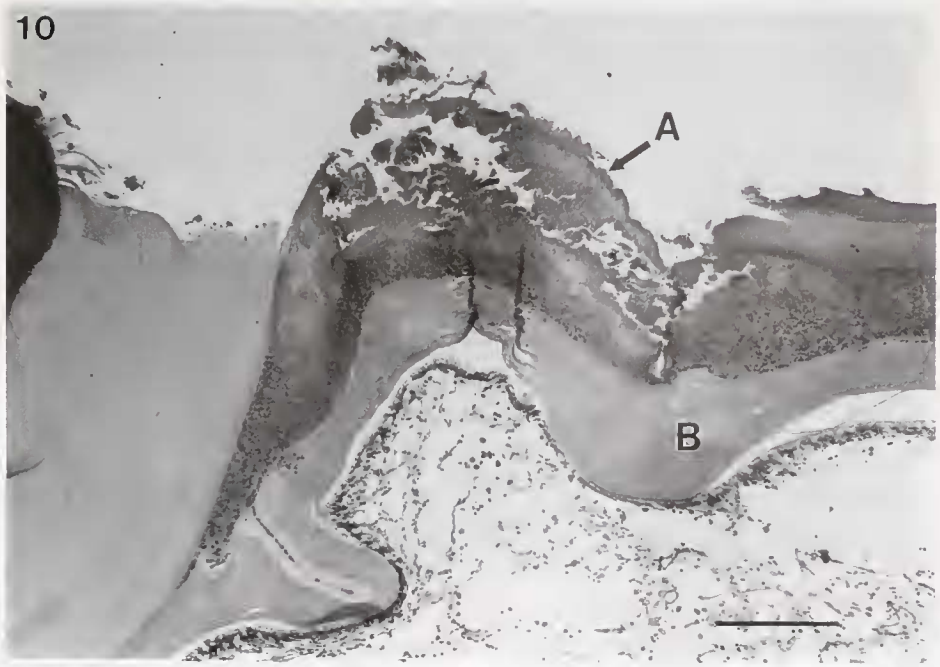

Figure 10. C_4/D_0, Grade IV. Melanization and necrosis of the exposed pseudomembrane (A). Increased depth of the inner thin-lamellar-endocuticle/membranous layer (B). Bar represents 220 μm.

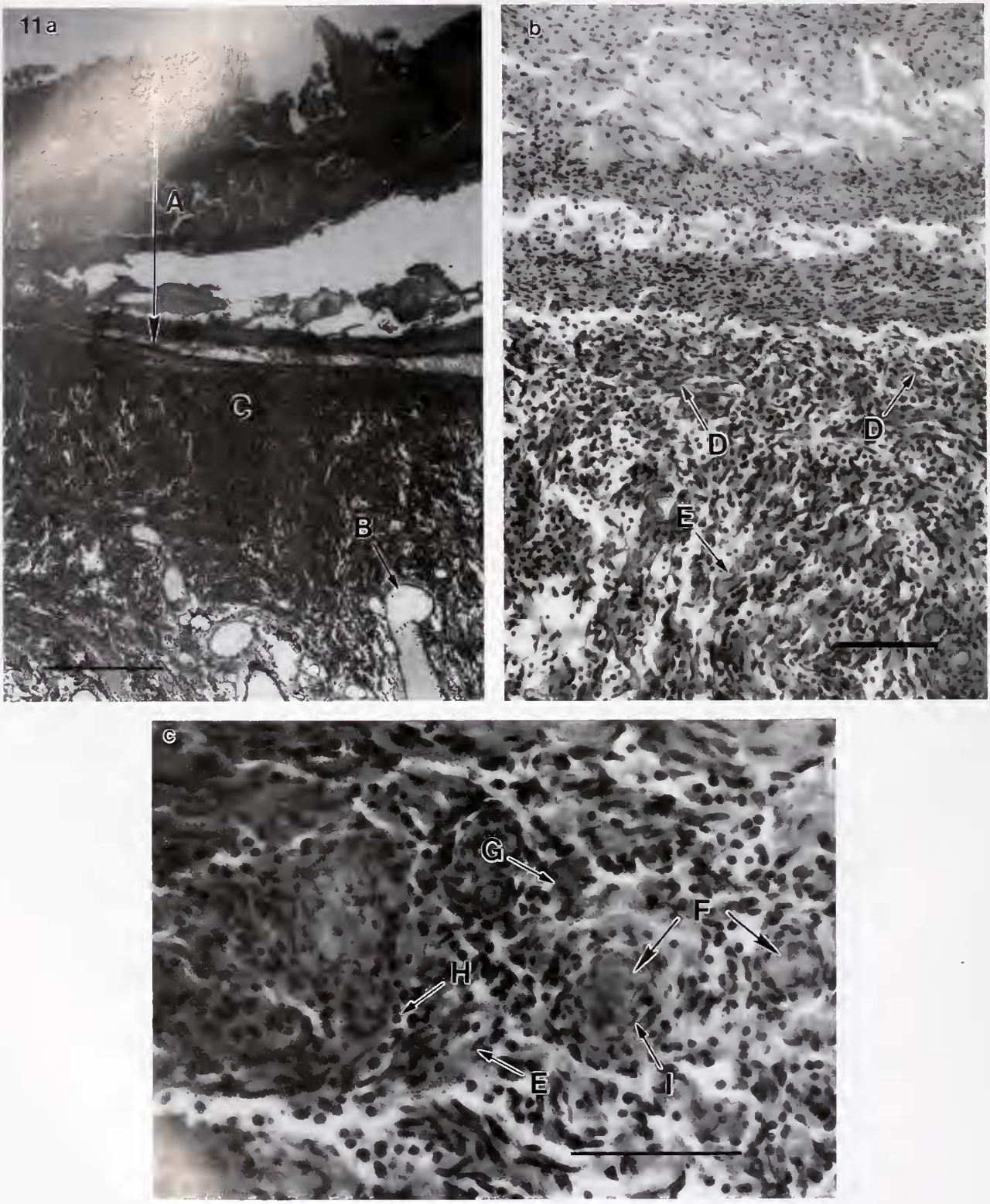

Figure 11. C_4/D_0, Grade V. 11a. Lamellae of transformed inflammatory cells alternating with necrotic debris at the surface (A) of the cuticular ulcer. Dilated vascular channels (B). Increase in cellularity of the dermis (C). Bar represents 620 μm. 11b. Transformed agranulocytes (D) occur at various angles to the ulcerated surface. Myoblastic proliferation is present in the deeper dermis (E). Bar represents 70 μm. 11c. Encapsulations (granulomas) (F) are present at various levels within the inflamed dermis containing untransformed inflammatory cells consisting of granulocytes (G) and agranulocytes/semigranulocytes (H) as well as transformed encapsulating inflammatory cells (I). Bar represents 64 μm.

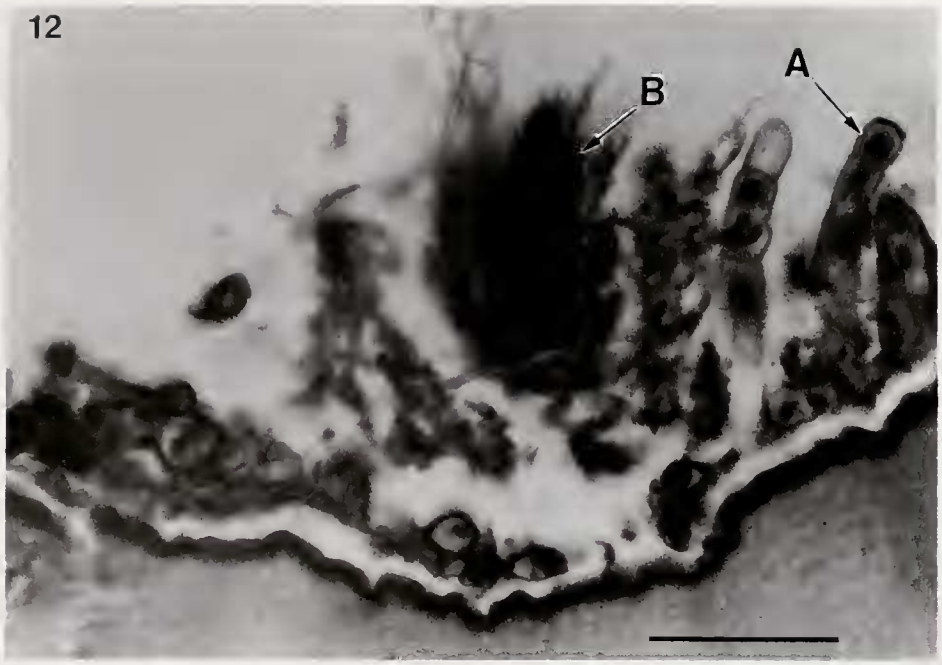

Figure 12. C_2/C_3, Grade I. Filamentous algae predominate (A) in the perisetal depression around the hair-fan organ (B). Bar represents 38 μm.

V lesion. In this lesion, a thick pseudomembrane, 100 or more cells in thickness, occurred below a necrotic, but still attached, thin-lamellar-endocuticle. No cuticular epithelium was present and the erosion was accompanied by deeper dermal inflammation similar to Grade V of C_4/D_0.

Discussion

Examination of homarid cuticular inflammation in winter impoundment shell disease revealed some important histological inflammatory mechanisms in all substages examined including: epicuticular deposition; melanization; inflammatory cell infiltration; and pseudomembrane formation. Proliferation of the membranous layer was seen only in those animals in C_4/D_0 of the molting cycle. Cuticular erosion development in the two molting substage groups, C_2/C_3 and C_4/D_0, was progressive. The erosions in both groups were divisible into grades, each of which demonstrated characteristic protective mechanisms.

In Grade 1 of C_2/C_3 and early C_4/D_0, epicuticle continued to be deposited, both within setal pits and throughout the cuticle, in an attempt to maintain the epicuticular layer and prevent erosion into the exocuticle. Although the continued production of epicuticle was only rarely confirmed histologically in this study, post-ecdysial production of this shellac-like cement layer by dermal tegmental glands has been previously confirmed in arthropods (Neville, 1975). However, the extent to which epicuticle was deposited varied with the activity of the tegmental glands as determined by the substage of the molting cycle (Arsenault *et al.*, 1979).

Filamentous algae predominated in early epicuticular erosions. The role these organisms play in the initial penetration of the epicuticle is not immediately obvious. Breaching of the epicuticle often occurred first at the shoulders and sides of the setal pits. In general, setal pits provided a protected cuticular microenvironment for colonization by fungi, and other organisms (Malloy, 1978). Erosions of higher grades were filled with a variety of microbes including fungi, bacteria, amoebae, and other protozoa. Chitinolytic and lipolytic bacteria were isolated in shell lesions from lobsters in all stages of the disease.

The cause of impoundment shell disease is not due to any single etiological agent, but rather results from the aggregate effects of epicellular enzymes produced by the mixture of various types of organisms present on the surface of the lesions (Sindermann, 1990). Swarming vibrios, pseudomonads, and *Flavobacter* spp. were cultured from the blood of five of eight lobsters. Bacteria of these types have previously been implicated in shell disease from a variety of marine crustacea (Sindermann *et al.*, 1989). Hemolymph infection by ciliated protozoa resembling *Mugardia* sp. (Sherburne and Bean, 1991) were noted histologically in sections of some severely affected animals. Indeed, severe shell lesions may have allowed for debabilitation of the lobsters, which increased the animals'

Figure 13. C_2/C_3. Grade II. Erosion and melanization (A) of the exocuticle is present. Mild accumulation of inflammatory cells (B) which appear to be predominately granulocytes at the base of the cuticle are often centered around cuticular canals (C). The epithelium is necrotic in one focus (D). Bar represents 60 μm.

susceptibility to opportunistic infections, such as that by ciliated protozoans.

Melanization was an important and prominent part of the inflammatory response and was seen in Grades I–V of both Stages C_4/D_0 and C_2/D_0. However, it was especially prominent in the striated cuticle of Grade III erosions. Melanized foci were bleached to varying degrees by the decalcification process and so appeared much lighter in sections than when viewed grossly (Smolowitz *et al.*, 1992).

Increased depth of the inner thin-lamellar-endocuticle/membranous layer in C_4/D_0 provided an excellent method for increasing the depth of the protective cuticular barrier between the cuticular epithelium and the environment. Interestingly, in addition to an increase in thickness of these layers, there is often a loss of the orderly deposition and distinctiveness of these two layers, which can be seen in histologically normal cuticle. The lack of orderly deposition is probably an indication of the similarity of the two layers and may reflect a vacillating cuticular secretory response by the cuticular epithelial cells to toxins, degradation products, etc. from the inflamed cuticle above or bacterial or protozoal septicemia. Proliferation of the inner thin-lamellar-endocuticle/membranous layer appeared to be distinct from new cuticle formation because dissolution of an ecdysial membrane [with retraction of the epithelium and formation of a molting space between the old cuticle and the cuticular epithelium (apolysis) and production of new epicuticle and exocuticle] was not evident (Travis, 1955; Skinner, 1962). In Stage C_2/C_3 of the molting cycle, the membranous layer was not present and the thin-lamellar-endocuticle alone did not proliferate in response to the inflammatory stimulus. This difference from substage C_4/D_0 may have been a result of the normal effects on the epithelial cells by the molting hormones (Neville, 1975).

Pathologic epithelial hypertrophy was noted in several lesions but was most consistently found from Grades III to V of all substages. In early C_2/C_3 and after late C_4/D_0, epithelial cells were physiologically hypertrophic diffusely throughout the cuticle indicating active cuticular secretion due to normal molting cycle activity. Thus in early C_2/C_3 and late C_4/D_0, pathological epithelial hypertrophy could not be differentiated from adjacent normal activity.

Formation of a pseudomembrane in Grade III of C_2/C_3 represents an important protective effort to prevent ulceration of the cuticular epithelium. Additionally, it is probable that continued secretion of cuticle would occur beneath the pseudomembrane by the still-intact epithelial cells upon appropriate stimulation perhaps by molting hormones. Thus, the inflammatory process could progress from Grade III of C_2/C_3 to Grade IV of C_4/D_0.

In C_4/D_0, the pseudomembrane is necessary for protection of the inner layers of the thin-lamellar-endocuticle/membranous layer so that separation of the epithelium at apolysis can still occur and prevent focal adhesion between the old and new shells. Pseudomembranes appear to develop progressively from foci that were at first identified as primarily granulocytic. However, the transformed cells responsible for formation of pseudomembranes appeared to originate from an influx of semigranulocytes or agranulocytes (Johnson, 1980; Johansson and Söderhäll, 1989). Inflammatory cell identification and function *in vivo* warrants further study. *In toto*, pseudomembranes probably represent the cuticular "clots" referred to in the literature (Bang, 1983; Sindermann *et al.*, 1989). The formation of pseudomembranes in Grade V of C_4/D_0 to shield the underlying dermis after epithelial ulceration, affords an additional method of inflammatory protection.

Dermal foci of encapsulation and melanization were common and probably reflected the vulnerability of teg-

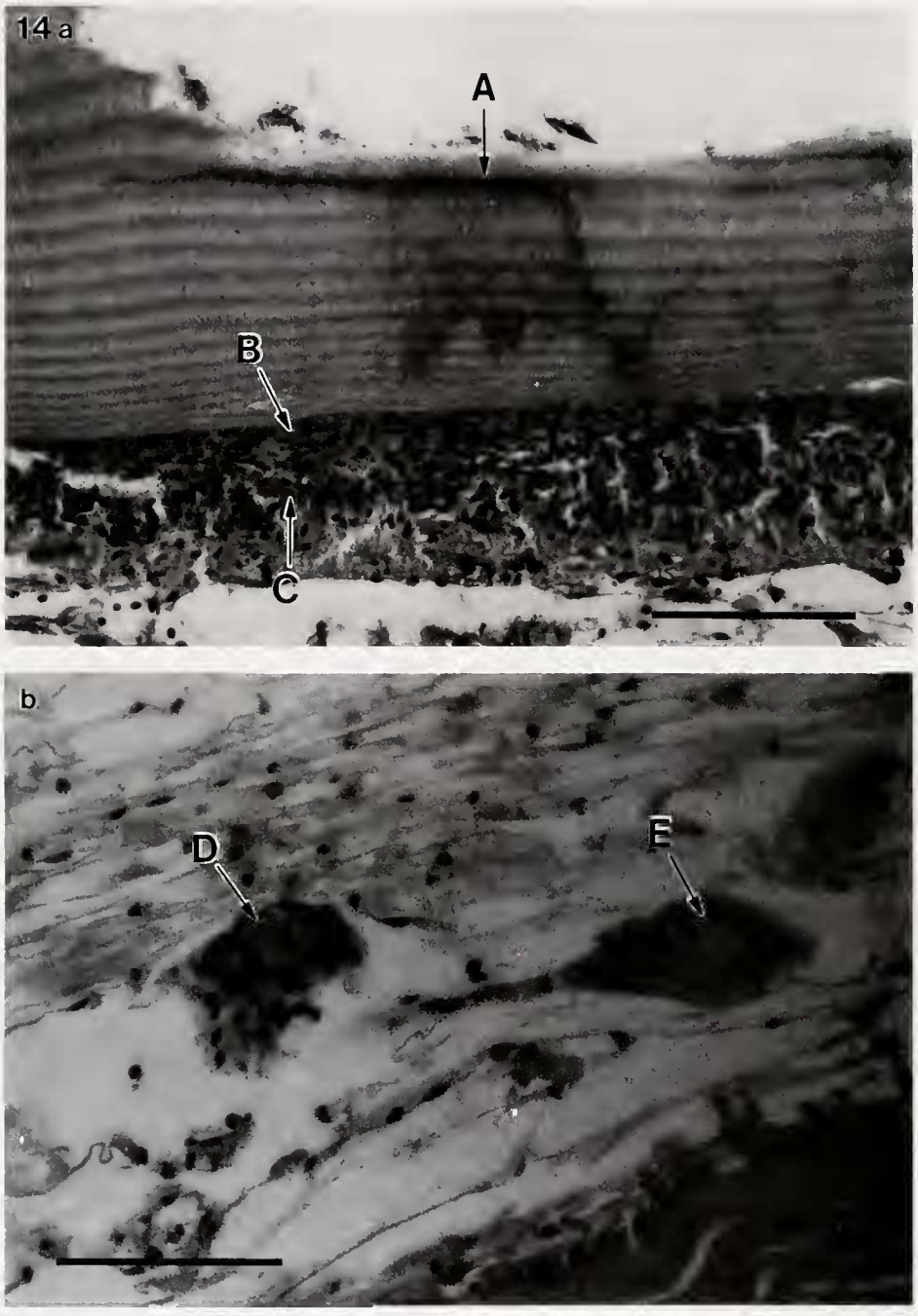

Figure 14. C_2/C_3, Grade III. 14a. Erosion into the striated endocuticle with melanization (A). Formation of a pseudomembrane (B) between the cuticular epithelium (C) and the base of the cuticle. Bar represents 120 μm. 14b. Inflamed tegmental gland with invading inflammatory cells (D) and adjacent normal tegmental gland (E). Bar represents 71 μm.

mental glands and setal cells to infection because they provide direct conduits to the surface by way of the cuticular canals. Other melanized encapsulations in the open portion of the circulatory system may have been related to stimulation of inflammatory cells by bacteremia or toxic products (Johansson and Söderhäll, 1989).

Examination of Grade III lesions in a molted shell and the postmolt cuticle demonstrate that molting can effectively eliminate lesions confined to the upper levels of the cuticle. However, molting is not completely effective if erosions through the pseudomembrane into the inner thin-lamellar-endocuticle/membranous layer have occurred or

cuticle has ulcerated. At this point, the ability to survive depends on a balance between numbers of infective organisms, husbandry conditions, and effectiveness of the inflammatory and reparative responses.

The inflammatory defense mechanisms of melanization, inner thin-lamellar-endocuticle/membranous layer proliferation, pseudomembrane proliferation, and molting provide homarids varied and extremely effective methods for preventing or inhibiting shell disease in their natural environment. However, inflammatory defense mechanisms do not seem as effective in protecting animals confined in stress-producing impoundment areas.

The classification scheme and description of cuticular inflammatory responses presented herein for winter impoundment shell disease can be used generally in situations with gradually developing cuticular lesions such as those due to pollution and overcrowding. Overlap between substages and grades should be expected because cuticle formation is not a static process and foci of erosion in cuticles can span several substages. However, this scheme provides a framework with which to begin the classification and further the understanding of these cuticular lesions. The function of inflammatory cells that seem to predominate in these erosions requires further study, especially in relation to the mediators produced and definite identification of the cells responsible for *in vivo* pseudomembrane formation and encapsulation. Future studies in our laboratory will include descriptions of the inflammatory responses in these and other stages of the molt cycle in relation to animals of varying ages, and development of a macroscopic impoundment shell disease grading system based on the histological grading system described in this paper. We also will study the differences between impoundment shell disease lesions and those due to other causes such as demonstrated in Figures 4 and 5. Comparative studies of cuticular inflammatory responses in other Crustacea are ongoing.

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